FACULTY OF **BIOSCIENCE ENGINEERING**

Stability of whey protein derived peptides upon severe protein glycation

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Introduction

Cow's milk and dairy products, major nutrients in the human diet, are important sources of **food allergens** and their extensive use in food products poses a serious threat to allergic consumers. Reliable **detection methods** are needed in order to ensure meticulous **labeling** and protect allergic consumers. Mass spectrometry is one of the dominant approaches used for the identification of proteins and peptides and is also gaining attention in the context of the food allergens detection. The aim of this study was to identify protein sequences of whey proteins, using **MALDI-TOF MS and MS/MS** analysis, despite the induced changes on molecular level as a consequence of a progressive **Maillard reaction**. These stable peptides hold potential to be used as analytical markers for the development of new quantitative methods for the detection of whey protein residues in processed foods.

Materials and methods

The model systems were obtained by mixing 1% (w/v) whey proteins with 6% (w/v) glucose. The mixtures were incubated at 70°C for up to 48 h. The model systems with soluble wheat proteins were obtained by mixing 0.25% (w/v) of whey proteins with 0.75% (w/v) of wheat proteins or 0.5% (w/v) whey proteins with 0.5% (w/v) wheat proteins and 6% (w/v) of glucose. Applied Biosystems MDS Sciex 4800 Plus MALDI TOF/TOF (USA) operating in reflectron positive ion mode was used for the peptide detection either directly after tryptic digestion, or with a previous separation of peptides on a reversed phase column. Protein identification was performed by searching in the protein sequence database (Swiss Prot) using the Mascot search engine (*http://www.matrixscience.com*).

Results and discussion

The interaction of glucose with proteins led to the formation of protein bound carbonyls, fluorescent compounds and losses of lysine residues next to the formation of high molecular weight aggregates (**Figure 1**). For native whey proteins, 6% sequence coverage was obtained for α-LA and 50% for β-LG. Due to the complex chemical modifications occurring during the Maillard reaction, a number of peptides present in the tryptic digest of the native whey proteins could not be observed in the digest of the glycated proteins. However, ^{57}Val – Lys^{76} and ^{31}Val – Arg^{56} peptides proved to remain stable even after 48h incubation with glucose either identified after separation on a RP column or directly by MALDI TOF MS and MS/MS (**Table 1 & Figure 2**). Peptides ^{57}Val – Lys^{76} and ^{31}Val – Arg^{56} were found in chocolate and cookie samples (**Table 2 & Figure 3**).

Expected $[M+H]^+$	Peptide	Measured mass $[M+H]^+$ Native	1% whey [a]	0.5% whey 0.5% wheat [a]	0.25% whey 0.75% wheat [a]
		α-LA			
1200.65	^{118}Val-Lys^{127}	1200.61(46)	1200.51 (42)	n/d	n/d
		β-LG			
1064.44	^{77}Trp-Lys^{85}	1064.39 (24)	n/d	n/d	n/d
1065.58	^{108}Val–Lys^{116}	1065.53 (64)	1065.46 (32)	n/d	n/d
1245.58	^{141}Thr–Lys^{151}	1245.52 (93)	1245.42 (21)	1245.59 (86)	1245.48 (87)
1658.78	^{165}Leu–Ile^{178}	1658.72 (40)	n/d	n/d	n/d
2313.25	^{57}Val–Lys^{76}	2313.19 (105)	2313.03 (52)	2313.00 (7)	2313.15 (115)
2707.37	^{31}Val–Arg^{56}	2707.30 (132)	2717.12 (68)	2707.10 (50)	2707.20 (88)

Table 1 *Peptides were separated on a RP column prior the MALDI-TOF MS analysis; in parenthesis assigned score: > 21 = significant homology; > 33 = identity or extensive homology (p<0.05); n/d – not detected, [a] – 48h incubated with 6% glucose at 70°C*

Expected $[M+H]^+$	Peptide	Chocolate	Cookie
	β-LG		
1245.58	^{141}Thr–Lys^{151}	1245.59 (78)	n/d
2313.25	^{57}Val–Lys^{76}	2313.28 (126)	+
2707.37	^{31}Val–Arg^{56}	2707.40 (223)	2707.34 (41)

Table 2 *Chocolate contained 7.5 mg whey proteins/g and cookies contained 1.3 mg whey proteins/g sample as theoretically calculated from the ingredient list*

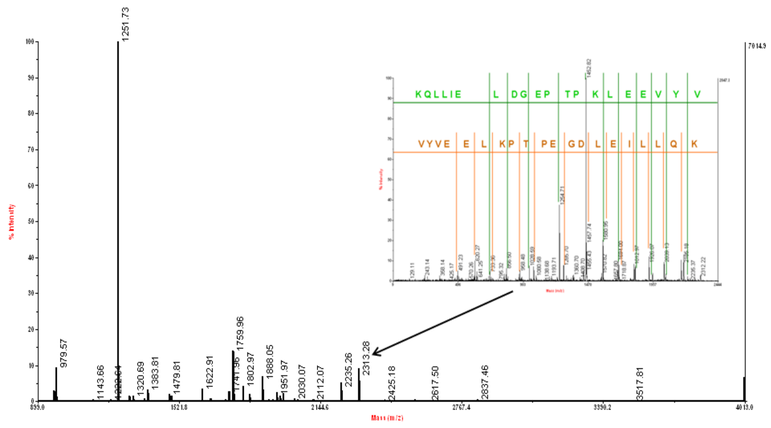

Figure 3 *MALDI-TOF MS and MS/MS spectra of the chocolate sample. The stable peptides ^{57}Val – Lys^{76} is marked with arrow. Matched Y- and b-ions are indicated in green and red respectively.*

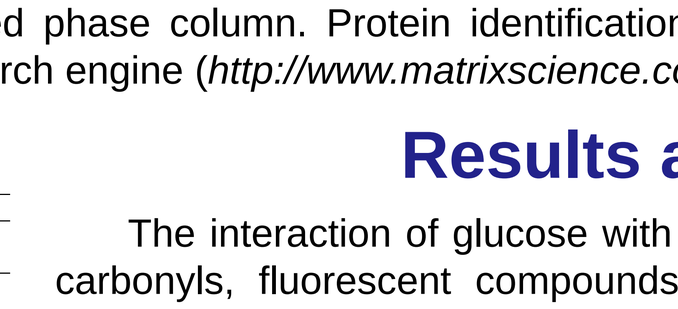

Figure 1 *SDS-PAGE pattern of whey proteins incubated in the presence or absence of wheat proteins with glucose*

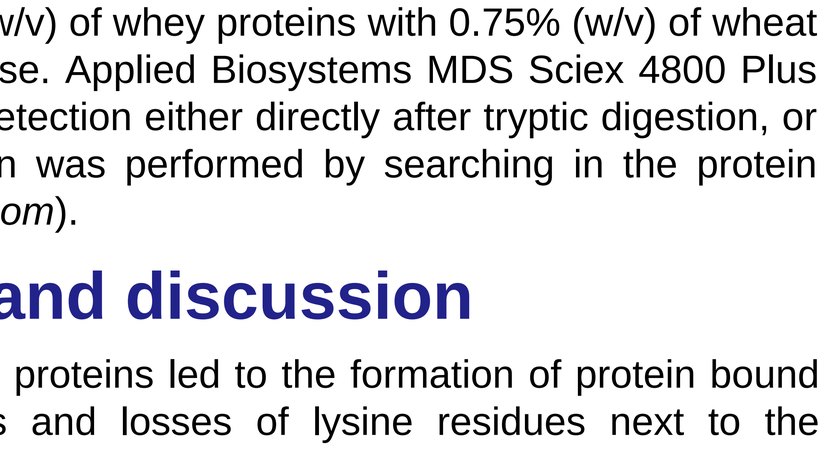

Figure 2 *MALDI-TOF MS spectra of native (A) and glycated whey proteins: B – 1% whey proteins 48h incubated with glucose; C – 0.25% whey with 0.75% wheat proteins 48h incubated with glucose. Peptides were analyzed directly via MALDI-TOF MS.*

Their presence, even if in samples initially having milk as ingredient and subjected to processing, indicates that they are stable not only under the studied food processing simulating conditions, but also in real food matrixes.

Conclusions

- Despite severe protein modifications induced by Maillard reaction, ^{57}Val – Lys^{76} and ^{31}Val – Arg^{56} β-LG derived peptides remained stable – can be used as analytical targets for the development of robust quantitative methods
- MALDI-TOF MS and MS/MS - useful tool for the screening of stable allergen derived peptides

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